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Photodynamic antibacterial effect of graphene quantum dots

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ABSTRACT

Synthesis of new antibacterial agents is becoming increasingly important in light of the emerging antibiotic resistance. In the present study we report that electrochemically produced graphene quantum dots (GQD), a new class of carbon nanoparticles, generate reactive oxygen species when photoexcited (470 nm, 1 W), and kill two strains of pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. Bacterial killing was demonstrated by the reduction in number of bacterial colonies in a standard plate count method, the increase in propidium iodide uptake confirming the cell membrane damage, as well as by morphological defects visualized by atomic force microscopy. The induction of oxidative stress in bacteria exposed to photoexcited GQD was confirmed by staining with a redox-sensitive fluorochrome dihydrorhodamine 123. Neither GQD nor light exposure alone were able to cause oxidative stress and reduce the viability of bacteria. Importantly, mouse spleen cells were markedly less sensitive in the same experimental conditions, thus indicating a fairly selective antibacterial photodynamic action of GQD.

1. Introduction

Different types of nanoparticles, ranging in size from 1 to 100 nm, have been investigated for their possible use in biomedicine [1]. Semiconductor quantum dots are nanoparticles with superior photo-physical properties suitable for biomedical imaging [2]. However, the potential toxicity resulting from the presence of heavy metal ions in conventional inorganic quantum dots (e.g. CdSe, CdTe) may impede their medical applications [3]. A new class of quantum dots, called graphene quantum dots (GQD), has recently been synthesized [4], displaying the special physico-chemical properties of graphene, a single layer of carbon atoms in a honeycomb structure, endowed with large surface area and excellent thermal/chemical stability [5]. Compared to conventional inorganic quantum dots, GQD possess several advantages, including ease of production, high fluorescent activity, resistance to photo-bleaching, excellent solubility and biocompatibility [4]. Because of these favorable features, GQD are more suitable candidates for non-toxic bioimaging or biosensing agents than their inorganic counterparts.

Despite similarities with semimetal graphene nanoparticles, semiconductor GQD, due to different electronic structure, display some unique physichochemical and biological properties. Unlike graphene and similarly to fullerenes (C_{60}), another carbon allotrope [6], GQD in suspension are able to generate reactive oxygen species (ROS) upon photoexcitation [7]. Therefore, GQD are potential candidates for photodynamic therapy, in which the light-excited compound kills cells by ROS generated through energy or electron transfer to molecular oxygen [8]. Accordingly, we have recently reported that GQD exposed to blue light kill cancer cells in a ROS-dependent manner [9]. Photodynamic therapy can also target microbial pathogens, including bacteria, which is becoming increasingly relevant in light of the emerging antibiotic resistance and consequent reduction in effectiveness of conventional therapy [10,11]. While most carbon-based nanomaterials, including fullerenes, carbon nanotubes and graphene display antibacterial properties [12–14], the effects of GQD on bacteria have not been investigated so far.

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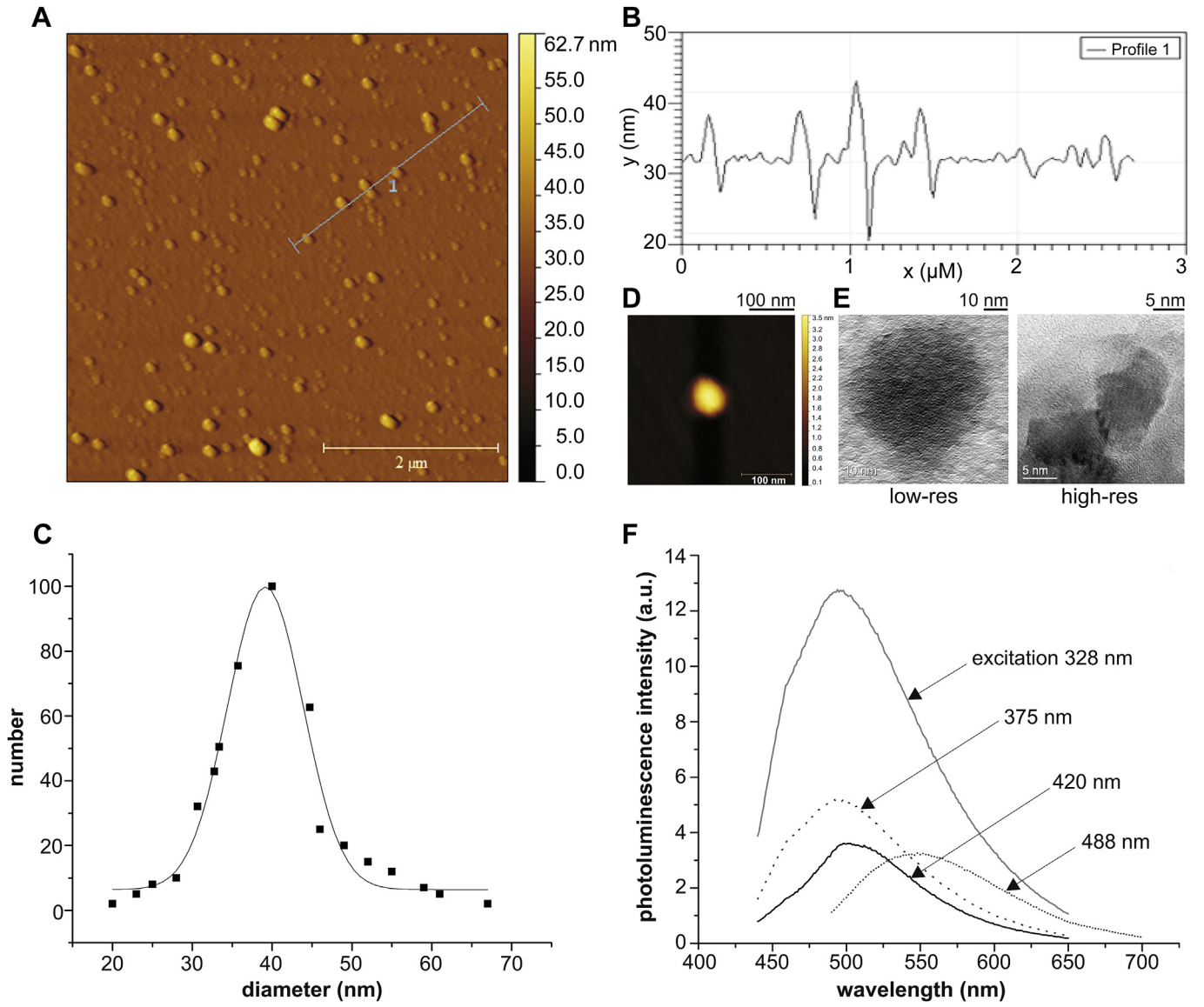

Fig. 1. Characterization of GQD. (A) Top view AFM image of GQD deposited on freshly cleaved mica substrate. (B) Height profile of GQD. (C) Size distribution of GQD nanoparticles calculated by AFM software ($n = 500$). (D) Top view AFM image of typical GQD. (E) Low- and high resolution-TEM micrographs of GQD. (F) Photoluminescence spectra of GQD exposed to different excitation wavelengths (a.u. – arbitrary units).

In the present study, we assessed photodynamic antibacterial activity of electrochemically produced GQD. To that aim, we used as targets methicillin-resistant *Staphylococcus aureus* (MRSA), Gram-positive cause of serious healthcare-associated and community-onset infections [15], and *Escherichia coli*, Gram-negative commensal of the human intestinal flora with pathogenic strains able to cause meningitis or urinary and gastrointestinal tract infections [16].

2. Materials and methods

2.1. Preparation and characterization of GQD

A stable suspension of GQD was prepared as previously described [9], using graphite rods as anode and cathode and NaOH/ethanol as electrolyte, followed by evaporation of the more volatile ethanol. The pH value of GQD suspension was adjusted to 7.0 by addition of hydrogen chloride and the total carbon particle and NaCl concentrations were adjusted to 1 mg/ml and 0.9%, respectively. We did not observe any visible aggregation of GQD in saline solution containing up to 5% of NaCl. A single GQD monolayer thin film was deposited on mica substrate (air-dried at 2000 °C for 10 min) by spin coating and imaged after drying by atomic force microscopy (AFM). AFM measurements were performed using a Quesant AFM (Aguora Hills, CA) operating in tapping mode in air on room temperature, with standard silicone tips (NanoAndMore Gmbh, Wetzlar, Germany) and with the constant force of 40 N/m. GQD were also characterized by transmission electron microscopy (TEM), using Philips CM200 microscope operated at 200 kV. Samples were prepared by drop casting of GQD dispersion on carbon coated copper grid with 300 mesh. The luminescence emission measurements were performed at room temperature on the Fluorolog-3 FL3-221 spectrofluorometer system (Horiba Jobin-Yvon S.A.S., Chilly Mazarin, France), utilizing a 450 W Xenon lamp as excitation source (328 nm) and R928P photomultiplier tube as a detector.

2.2. Bacterial suspension and treatment

Stock cultures of a clinical isolate of MRSA [17] and the reference strain of *E. coli* (ATCC25922) were maintained on Columbia agar (BD, Franklin Lakes, NJ) supplemented with 5% sheep blood at 4 °C. Prior to inoculation, the strains were transferred from the stock cultures to Columbia agar supplemented with 5% sheep blood and incubated aerobically at 37 °C overnight, followed by subcultivation under the same conditions. The cultures were then used for preparation of bacterial suspensions (2×10^4 colony forming units/ml) in a phosphate-buffered saline (PBS). Subsequently, 200 μl of bacterial suspension were transferred to 15 ml glass centrifuge tube and 200 μl of GQD (final concentration 50–200 μg/ml) or PBS were added. After irradiation with blue light (465–475 nm, 1 W), bacterial suspensions were centrifuged at 4000 g for 10 min and resuspended in PBS for determination of cell death/membrane damage, ROS measurement or AFM analysis.